AMENDMENT

In the Claims:

1. (Currently amended) A method for preparing an improved nucleic acid ligand, said nucleic acid ligand capable of binding to and altering the function of a target molecule, wherein said improvement is produced by modifying a nucleic acid ligand to have enhanced target function altering activity, said method comprising:

a) contacting a candidate mixture with the target, wherein nucleic acids having an increased affinity to the target relative to the candidate mixture may be partitioned from the remainder of the candidate mixture;

b) partitioning the increased affinity nucleic acids from the remainder of the candidate mixture;

c) amplifying the increased affinity nucleic acids to yield a ligand-enriched mixture of nucleic acids;

d) repeating steps a)-c) as necessary to identify a nucleic acid ligand of said target;

e) determining the target function altering activity of said nucleic acid ligand of step d);

f) modifying the nucleic acid ligand of step d), wherein said modifying comprises adding, deleting, or substituting nucleotide residues, wherein said residues may be chemically modified, and/or chemically modifying said nucleic acid ligand; and

g) determining whether the modified nucleic acid ligand of step f) has enhanced target function altering activity relative to the target function altering activity of the nucleic acid ligand of step ~~d)~~e), whereby said improved nucleic acid ligand may be prepared.

2. (Original) The method of claim 1 wherein the target function altering activity is inhibition of target function.

3. (Original) The method of claim 1 wherein the target function altering activity is augmentation of target function.

4. (Original) An improved nucleic acid ligand identified according to the method of claim 1.

5. (Original) The method of claim 1 wherein step f) comprises preparing a modified nucleic acid ligand that is identical to the nucleic acid ligand of step d) except for a single residue substitution.

6. (Currently amended) The method of ~~clam~~ claim 1 wherein step f) comprises preparing a modified nucleic acid ligand and that is identical to the nucleic acid ligand of step d) except for the absence of one or more terminal residues.

7. (Original) The method of claim 1 wherein step f) comprises preparing a modified nucleic acid ligand by chemically modifying the nucleic acid ligand of step d).

8. (Original) The method of claim 1 wherein in step f) the nucleic acid ligand of step d) is modified while in contact with the target.

9. (Currently amended) A method for preparing an improved nucleic acid ligand, said nucleic acid ligand capable of binding to and altering the function of a target molecule, wherein said improvement is produced by modifying a nucleic acid ligand to have enhanced target function altering activity, said method comprising:

a) contacting a candidate mixture with the target, wherein nucleic acids having an increased affinity to the target relative to the candidate mixture may be partitioned from the remainder of the candidate mixture;

b) partitioning the increased affinity nucleic acids from the remainder of the candidate mixture;

c) amplifying the increased affinity nucleic acids to yield a ligand-enriched mixture of nucleic acids;

d) repeating steps a)-c) as necessary to identify a nucleic acid ligand of said target;

e) determining the target function altering activity of said nucleic acid ligand of step d);

f) determining the three-dimensional structure of said nucleic acid ligand, wherein said determining is selected from the group consisting of determining which nucleic acid residues in a nucleic acid ligand are necessary for maintaining the three-dimensional structure of the ligand, which residues interact with the target to facilitate the formation of ligand-target binding pairs, and both determining which nucleic acid residues in a nucleic acid ligand are necessary for maintaining the three-dimensional structure of the ligand and which residues interact with the target to facilitate the formation of ligand-target binding pairs;

g) modifying the nucleic acid ligand of step d) based on the determined three-dimensional structure of the nucleic acid ligand, wherein said modifying comprises adding, deleting, or substituting nucleotide residues, wherein said residues may be chemically modified, and/or chemically modifying said nucleic acid ligand; and

h) determining whether the modified nucleic acid ligand of step g) has enhanced target function altering activity relative to the target function altering activity of the nucleic acid ligand of step ~~d)~~e), whereby said improved nucleic acid ligand may be prepared.

10. (Original) The method of claim 9 wherein the target function altering activity is inhibition of target function.

11. (Original) The method of claim 9 wherein the target function altering activity is augmentation of target function.

12. (Original) An improved nucleic acid ligand identified according to the method of claim 9.

13. (Original) The method of claim 9 wherein in step f) the three-dimensional structure of a nucleic acid ligand is determined by:

i) chemically modifying denatured and nondenatured nucleic acid ligand; and

ii) determining which nucleotide residues are modified in the denatured nucleic acid ligand that are not modified in the nondenatured nucleic acid ligand.

14. (Original) The method of claim 9 wherein in each of steps d)-f), said nucleic acid ligand comprises a plurality of nucleic acid ligands, and in step f) the three-dimensional structure of the nucleic acid ligands is determined by covariance analysis on said plurality of nucleic acid ligands of step d).

15. (Currently amended) A method for designing an improved nucleic acid ligand to a given target from a plurality of nucleic acid ligands to said target, said nucleic acid ligands capable of binding to and altering the function of said target, wherein said improved nucleic acid ligand has enhanced target function altering activity, and wherein said designing is accomplished by the method comprising:

a) determining the three dimensional structure of said nucleic acid ligands;

b) determining the nucleic acid residues of said nucleic acid ligands that are responsible for altering the function of said target, wherein said determining is selected from the group consisting of determining which nucleic acid residues in a nucleic acid ligand are necessary for maintaining the three-dimensional structure of the ligand, which residues interact with the target to facilitate the formation of ligand-target binding pairs, and both determining which nucleic acid residues in a nucleic acid ligand are necessary for maintaining the three-dimensional structure of the ligand and which residues interact with the target to facilitate the formation of ligand-target binding pairs; and

c) designing said modified nucleic acid ligand to said target by modifying said nucleic acid ligand by adding, deleting, or substituting nucleotide residues based on said determinations made in steps a) and b) and wherein said added or substituted nucleotide residues may be chemically modified.

16. (Original) The method of claim 15 wherein the target function altering activity is inhibition of target function.

17. (Original) The method of claim 15 wherein the target function altering activity is augmentation of target function.

18. (Original) An improved nucleic acid ligand identified according to the method of claim 15.

19. (Currently amended) A method for designing an improved nucleic acid ligand, said nucleic acid ligand capable of binding to and altering the function of a given target, wherein said improvement is produced by modifying a nucleic acid to have enhanced target function altering activity, said method comprising:

a) contacting a candidate mixture of nucleic acids with the target, wherein nucleic acids having an increased affinity to the target relative to the candidate mixture may be partitioned from the remainder of the candidate mixture;

b) partitioning the increased affinity nucleic acids from the remainder of the candidate mixture;

c) amplifying the increased affinity nucleic acids to yield a ligand-enriched mixture of nucleic acids;

d) repeating steps a)-c) as necessary to identify a nucleic acid ligand of said target;

e) determining the target function altering activity of said nucleic acid ligand of step d);

f) determining the three-dimensional structure of said nucleic acid ligand;

g) determining the nucleic acid residues of the nucleic acid ligand that are responsible for the target function altering activity of said nucleic acid ligand, wherein said determining is selected from the group consisting of determining which nucleic acid residues in a nucleic acid ligand are necessary for maintaining the three-dimensional structure of the ligand, which residues interact with the target to facilitate the formation of ligand-target binding pairs, and both determining which nucleic acid residues in a nucleic acid ligand are necessary for maintaining the three-dimensional structure of the ligand and which residues interact with the target to facilitate the formation of ligand-target binding pairs; and

h) designing said improved nucleic acid ligand to said target by modifying the nucleic acid ligand by adding, deleting, or substituting nucleotide residues based on said determinations made in steps f) and g), and wherein said added or substituted residues may be chemically modified.

20. (Original) The method of claim 19 wherein the target function altering activity is inhibition of target function.

21. (Original) The method of claim 19 wherein the target modulating activity is augmentation of target function.

22. (Original) An improved nucleic acid ligand identified according to the method of claim 19.

23. (Original) The method of claim 1 wherein step f) comprises substitution of chemically modified nucleotides in the nucleic acid ligand of step d).

24. (Original) The method of claim 23 wherein said chemically modified nucleotides are selected from the group consisting of 5-position modified pyrimidines, 8-position modified purines, 2'-modified nucleotides or combinations thereof.

25. (Original) The method of claim 23 wherein step f) comprises the substitution of a 2'-modified nucleotide for its respective 2'-OH nucleotide in the nucleic acid ligand of step d).

26. (Original) The method of claim 25 wherein said 2'-modified nucleotide is selected from the group consisting of 2'-F nucleotides, 2'-NH_2 nucleotides, and 2'-O-Methyl nucleotides.

27. (Original) The method of claim 1 wherein step f) comprises the addition of nucleotides to the 5' end of the nucleic acid ligand of step d), the 3' end of the nucleic acid ligand of step d), or both.

28. (Currently amended) A method for designing an improved nucleic acid ligand of a given target from a plurality of unimproved nucleic acid ligands to said target, said nucleic acid ligands capable of binding to and altering the function of said target, wherein said improved nucleic acid ligand has enhanced target function altering activity relative to unimproved nucleic acid ligands, and wherein said designing is accomplished by the method comprising:

a) contacting a candidate mixture with a target, wherein nucleic acids having an increased affinity to the target relative to the candidate mixture may be partitioned from the remainder of the candidate mixture;

b) partitioning the increased affinity nucleic acids from the remainder of the candidate mixture;

c) amplifying the increased affinity nucleic acids to yield a ligand-enriched mixture of nucleic acids;

d) repeating steps a)-c) as necessary to identify said plurality of unimproved nucleic acid ligands;

e) determining the target function altering activity of said plurality of unimproved nucleic acid ligands;

f) determining the consensus primary structure of said unimproved nucleic acid ligands;

g) designing an improved nucleic acid ligand by adding random a sequence of nucleotide residues to the minimal consensus primary structure, deleting~~,~~ or substituting nucleotide residues ~~based on said~~ not clearly conserved in said consensus primary structure, wherein said residues may be chemically modified~~, based on the determined consensus primary structure of the nucleic acid ligands of step d)~~; and

h) determining whether the improved nucleic acid ligand of step g) has altered target modulatory activity relative to the target function altering activity of the nucleic acid ligands of step ~~d)~~e), whereby said improved nucleic acid ligand may be designed.

29. (Original) The method of claim 28 wherein the target function altering activity is inhibition of target function.

30. (Original) The method of claim 28 wherein the target modulating activity is augmentation of target function.

31. (Original) An improved nucleic acid ligand identified according to the method of claim 28.

32. (Original) The method of claim 28 wherein said improved nucleic acid ligand of step g) is designed by deleting a portion of the nucleotides that are not part of the consensus primary structure.

33. (Currently amended) A method for designing an improved nucleic acid ligand of a given target from a plurality of unimproved nucleic acid ligands to said target, said nucleic acid ligands capable of binding to and altering the function of said target, wherein said improved nucleic acid ligand has enhanced target function altering activity relative to unimproved nucleic acid ligands, and wherein said designing is accomplished by the method comprising:

a) contacting a candidate mixture with a target, wherein nucleic acids having an increased affinity to the target relative to the candidate mixture may be partitioned from the remainder of the candidate mixture;

b) partitioning the increased affinity nucleic acids from the remainder of the candidate mixture;

c) amplifying the increased affinity nucleic acids to yield a ligand-enriched mixture of nucleic acids;

d) repeating steps a)-c) as necessary to identify said plurality of unimproved nucleic acid ligands;

e) determing the target ~~modulatory~~ function altering activity of said plurality of unimproved nucleic acid ligands;

f) determining the consensus secondary structure of said unimproved nucleic acid ligands;

g) designing an improved nucleic acid ligand by adding, deleting, or substituting nucleotide residues from a consensus loop, or consensus bulge, or a consensus stem, including pairs of base-paired nucleotide residues in a consensus stem~~based on said consensus secondary structure~~, wherein said residues may be chemically modified, based on the determined consensus secondary structure of the nucleic acid ligands of step d); and

h) determining whether the improved nucleic acid ligand of step g) has enhanced target function altering activity relative to the target function altering activity of the nucleic acid ligands of step ~~d)~~e), whereby said improved nucleic acid ligand may be designed.

34. (Original) The method of claim 33 wherein the target function altering activity is inhibition of target function.

35. (Original) The method of claim 33 wherein the target modulating activity is augmentation of target function.

36. (Original) An improved nucleic acid ligand identified according to the method of claim 33.

37. (Original) The method of claim 33 wherein said improved nucleic acid ligand of step f) is designed by deleting a portion of the nucleotides that are not part of the consensus secondary structure.

38. (Currently amended) The method of claim 33 wherein said nucleic acid ligand in step f) comprises a plurality nucleic acid ligands and the consensus secondary structure is determined by covariance analysis.

39. (Currently amended) A method for preparing an improved nucleic acid ligand, said nucleic acid ligand being a ligand of an intracellular target, wherein said improvement is produced by modifying a nucleic acid ligand to have enhanced cell membrane permeability, comprising:

a) contacting a candidate mixture of ~~of~~ nucleic acids with the target, wherein nucleic acids having an increased affinity to the target relative to the candidate mixture may be partitioned from the remainder of the candidate mixture;

b) partitioning the increased affinity nucleic acids from the remainder of the candidate mixture;

c) amplifying the increased affinity nucleic acids to yield a ligand-enriched mixture of nucleic acids;

d) repeating steps a)-c), as necessary, to identify a nucleic acid ligand;

e) determining the cell membrane permeability of said nucleic acid ligand;

~~e)~~f)modifying the nucleic acid ligand of step d), wherein said modifying comprises adding, deleting, or substituting nucleotide ~~said~~ residues, wherein said residues may ~~may~~ be chemically modified, and/or chemically modifying said nucleic acid ligand; and

~~f)~~g)determining whether the modified nucleic acid ligand of step e) has increased cell membrane permeability relative to the cell membrane permeability of the nucleic acid ligand of step ~~d)~~e) whereby said improved nucleic acid ligand may be prepared.

40. (Original) An improved nucleic acid ligand identified according to the method of claim 40.